The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89 (2), Page 7709- 7713

Does Fibrinogen Like Protein 2 Play A Role in High-Grade Glioma as A Prognostic Factor?

Sohaila Essam Mohamed*[1], Rania Makboul[2], Ahmed A. S. Elhakeem[3], Wael M. A. Abd El-Ghani[4], Mohamed Alaa Mohamed[1], Samir Eid[1], Maha Elnaggar[1]

Departments of [1]Oncology, [2]Pathology and [4]Neurosurgery,
Faculty of Medicine, Assiut University, Egypt, Assiut, Egypt
Department of [3]Pathology, Faculty of Medicine, Al-Azhar University, Assiut, Egypt
***Corresponding author:** Sohaila Essam Mohamed, **Mobile**: (+20) 01064509619; **Email:** sohailaessam2019@gmail.com

ABSTRACT

Background: Clotting fibrinogen-like protein 2 (FGL2) is a member of the fibrinogen-related protein family, FGL2 accelerated the development of glioblastoma multiforme (GBM) by triggering several immunosuppressive processes. **Objective:** The current study intended to understand FGL2 expression and prognostic significance in patients with high-grade gliomas. **Patients and Methods:** In our study, we examined 60 samples from patients with high-grade gliomas who received temozolomide and radiation at the same time from October 2019 to December 2020, as well as a one-year follow-up. FGL2 immunohistochemistry is used to measure the amount of FGL2. **Result:** Those who had progressed had considerably greater FGL2 percentages (76.54 18.52 vs. 56.17 33.41(%); P=0.04) than patients who had not progressed. Patients whose illness was progressing had higher levels of FGL2. Negative FGL2 expression was present in seven (14.9%) individuals who had no illness progression. Patients with negative FGL2 and mild intensity had the highest disease-free progression (DFP) (25 months), whereas those with strong intensity had the lowest (14 months). Among patients who passed away, the percentage of FGL2 was substantially greater (64.86 31.58 vs. 54.16 31.71(%); P 0.03). Patients with negative FGL2 and mild intensity had the highest DFP, whereas those with strong intensity had the lowest overall survival (19 months) (27 and 26 months, respectively). FGL2 at a cut-off point > 70% had 67% sensitivity and 61% specificity with an overall accuracy of 63.4% for predicting death in individuals with high-grade glioma. **Conclusion:** FGL2 expression in high-grade glioma patients can be utilized as a prognostic indicator, though further research is needed to fully understand the predictive usefulness.

Keywords: Fibrinogen-like protein 2; High-grade glioma; Disease free progression.

INTRODUCTION

Adult brain tumors with gliomas are the most prevalent; each year, about 10,000 new cases are evaluated [(1)]. The prognosis remains poor, with a survival of 12 to 15 months even after concurrent and adjuvant temozolomide, radiation, and the maximum safe resection [(2)]. Both innate and adaptive immunity are regulated by fibrinogen-like protein 2 (FGL2), which was discovered using microarray analytic screening as a potential candidate gene influencing Treg function [(3, 4)]. By boosting the expression of ectonucleoside triphosphate diphosphohydrolase 1 and programmed cell death protein 1 (PD-1), high levels of FGL2 expression in glioblastoma cause immunosuppression. As a result, FGL2 is a focal point for immunosuppression caused by glioblastoma, albeit it is unknown whether it can promote the transformation of LGG into HGG [(5, 6)].

The current study's objective was to assess FGL2 prognostic usefulness in people with high-grade gliomas.

PATIENTS AND METHODS

Study design and setting;

At the laboratory of Assiut University Hospital, cross-sectional research was done. The study included 60 samples from patients who were more than 18 years old, had a pathologically confirmed high-grade glioma, underwent whole or partial resection of the tumor, got concurrent radiation treatment with temozolomide, and then received adjuvant temozolomide for six months. Pregnancy, nursing, other comorbidities, a history of cancer, radiation therapy, or chemotherapy were among the exclusion criteria.

Methods

This study comprised sixty specimens of glioblastoma referred to the AUH laboratory. After examination of hematoxylin& eosin (H&E) slides of all glioblastoma specimens; we selected the slides of the cases with available follow-up data. Histological diagnosis of H&E stained sections had been reviewed and the histological prognostic criteria have been assessed.

Mouse monoclonal antibodies are utilized in immunohistochemical staining for FGL2, and immunoperoxidase staining is used to visualize the results. Dilutions of the antibodies, antigen retrieval techniques, and incubation periods were carried out following the manufacturer's instructions.

Ethical consideration:

The academic and ethical committee at Assiut University approved the study. All participants were having signed informed consent after being told of the study's aim. The Declaration of Helsinki, the World Medical Association's code of ethics for studies involving humans, guided the conduct of this work. ClinicalTrials.gov Identifier: NCT04113278.

Statistical analysis

SPSS was used to gather and analyze the data (Statistical Package for the Social Science, version 20,

Received: 15/7/2022
Accepted: 18/9/2022

IBM, and Armonk, New York). The mean ± standard deviation (SD) of quantitative data was expressed and compared using the Student t-test. Numbers (n) and percentages (%) are used to represent nominal data. Such data were subjected to the Chi^2 test. The current study used Kaplan Meier analysis to estimate overall survival and disease-free progression. The receiver operator characteristic curve was used to evaluate the diagnostic accuracy of FGL2 in predicting mortality and progression among patients with high-grade gliomas. Therefore, the level of confidence was maintained at 95%. P value was deemed significant if it was less than 0.05.

RESULTS

Baseline data of enrolled patients (Table 1):

The mean age of enrolled patients was 48.88 years with a range between 19 and 68 years. The majority (75%) of patients were males and 15 (25%) patients were females. As regards one-year follow-up, it was found that 20 (33.3%), 33 (55%), and 7 (11.7%) patients had a regressive disease, stationary disease, and progressive disease, respectively.

Table (1): Baseline data of enrolled patients

	N= 60
Age (years) Range	48.88 ± 12.45 19-68
Sex Male Female	 45 (75%) 15 (25%)
Long axis (cm)	4.58 ± 1.10
Site of lesion	
Parietal lobe	17 (28.3%)
Temporal lobe	13 (21.7%)
Tempo-parietal lobe	7 (11.7%)
Frontal lobe	6 (10%)
Occipital lobe	6 (10%)
Corpus callosum	5 (8.3%)
Posterior fossa	2 (3.3%)
Other areas	4 (6.7%)
Effect of the mass	
Minimal edema	12 (20%)
Mild edema	43 (71.7%)
Moderate edema	3 (5%)
Midline shift	46 (58.3%)
Compression of ventricle	26 (43.3%)
Concurrent temodal	59 (98.3%)
Adjuvant temodal	47 (78.3%)
2nd line chemotherapy	9 (15%)
2nd surgery	8 (13.3%)
2nd radiotherapy	2 (3.3%)
Mid-cycle assessment	
Regressive disease	20 (33.3%)
Stationary disease	33 (55%)
Progressive disease	7 (11.7%)

Data expressed as frequency (percentage), mean (SD)

Survival analysis among enrolled patients (Table 2):

Out of the studied patients; 24 (40%) patients were alive while 36 (60%) patients deteriorated and died. Overall survival was 26 months with a 95% CI between 25 and 35.

Table (2): Survival analysis among enrolled patients

	N= 60
Outcome Alive Died	24 (40%) 36 (60%)
Overall survival Month (95%CI)	26 (25-35)
Progression	13 (21.7%)
Progression-free survival Month (95%CI)	18 (15-20)

CI: confidence interval

Fibrinogen-like protein2 among enrolled patients (Table 3):

FGL2 has a mean of 61±15.00 (%) with a 95% to 3% range. Seven (11.7%) patients were reported to have negative FGL2 results. 16 (26.7%), 20 (33.3%), and 17 (28.3%) of the patients had low, moderate, and strong FGL2 intensity, respectively.

Table (3): Fibrinogen-like protein2 among enrolled patients

	N= 60
FGL2 (%) Range	61 ± 15.00 3-95
Intensity of FGL2 Negative Mild Moderate Strong	 7 (11.7%) 16 (26.7%) 20 (33.3%) 17 (28.3%)

Data expressed as frequency (percentage), and mean (SD).
FGL2: fibrinogen-like protein 2.

Disease-free progression based on the intensity of FGL2 (Table 4, Figure 1):

Increases in FGL2 intensity resulted in a considerable slowing in disease-free progression, with patients who experienced the strongest intensities experiencing the lowest DFP (14 months).

Table (4): Disease-free progression based on the intensity of FGL2

	Progression-free survival
Intensity of FGL2 Negative Mild Moderate Strong	 25 (23-42) 25 (23-42) 21 (25-27) 14 (8-19)
P-value	**< 0.001**

Data expressed as mean (95% confidence interval). P value was significant if < 0.05. FGL2: fibrinogen-like protein-2.

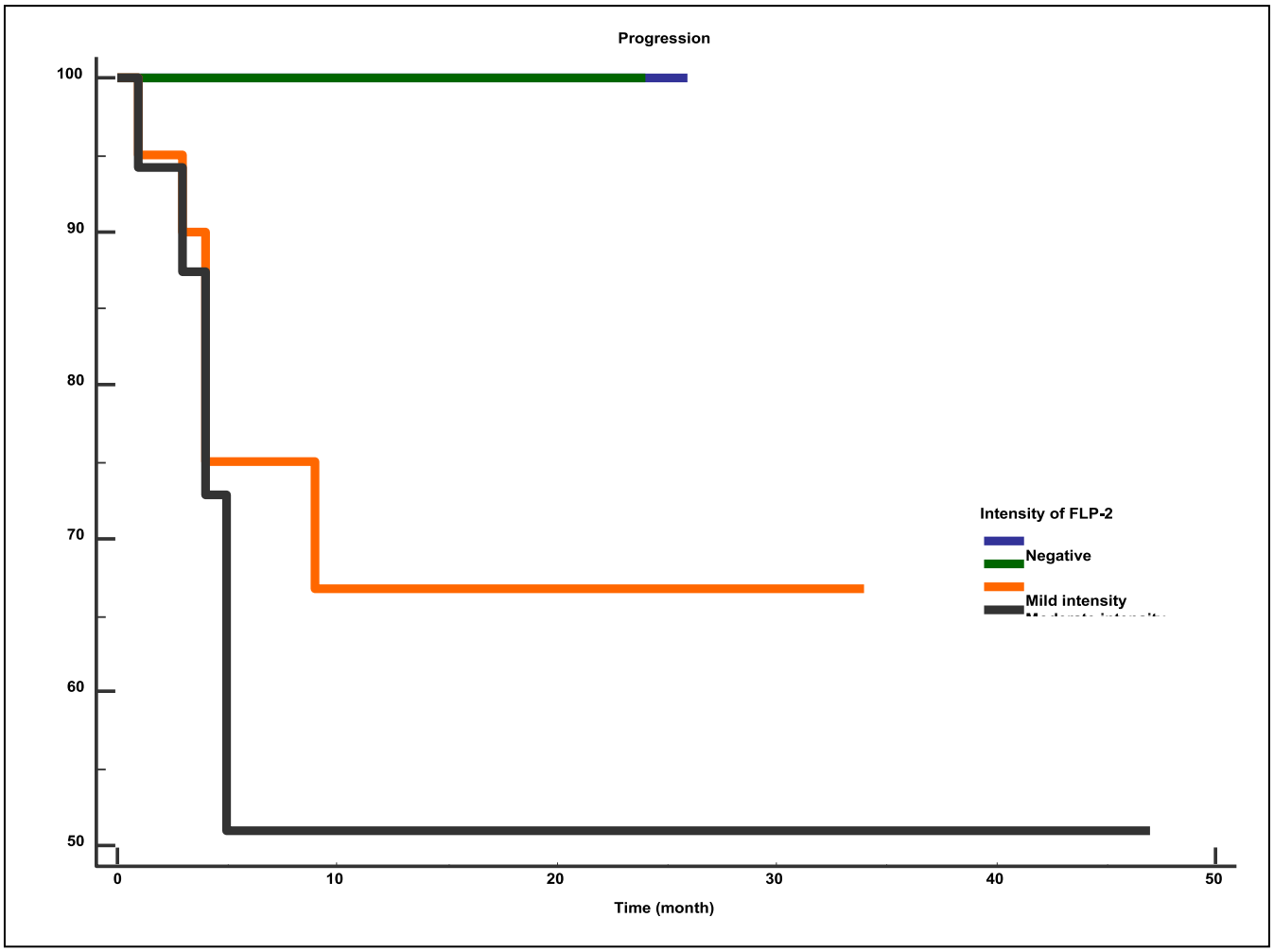

Figure (1): Disease-free progression based on the intensity of FGL2

Overall survival based on the intensity of FGL2 (Table 5, Figure 2):
Overall survival was significantly decreased with an increase in the intensity of FGL2.

Table (5): Overall survival based on the intensity of FGL2

	Overall survival
Intensity of FGL2	
Negative	27 (21-30)
Mild	26 (19-29)
Moderate	22 (18-29)
Strong	19 (10-28)
P-value	**< 0.001**

Data expressed as mean (95% confidence interval). P value was significant if < 0.05. FGL2: fibrinogen-like protein-2

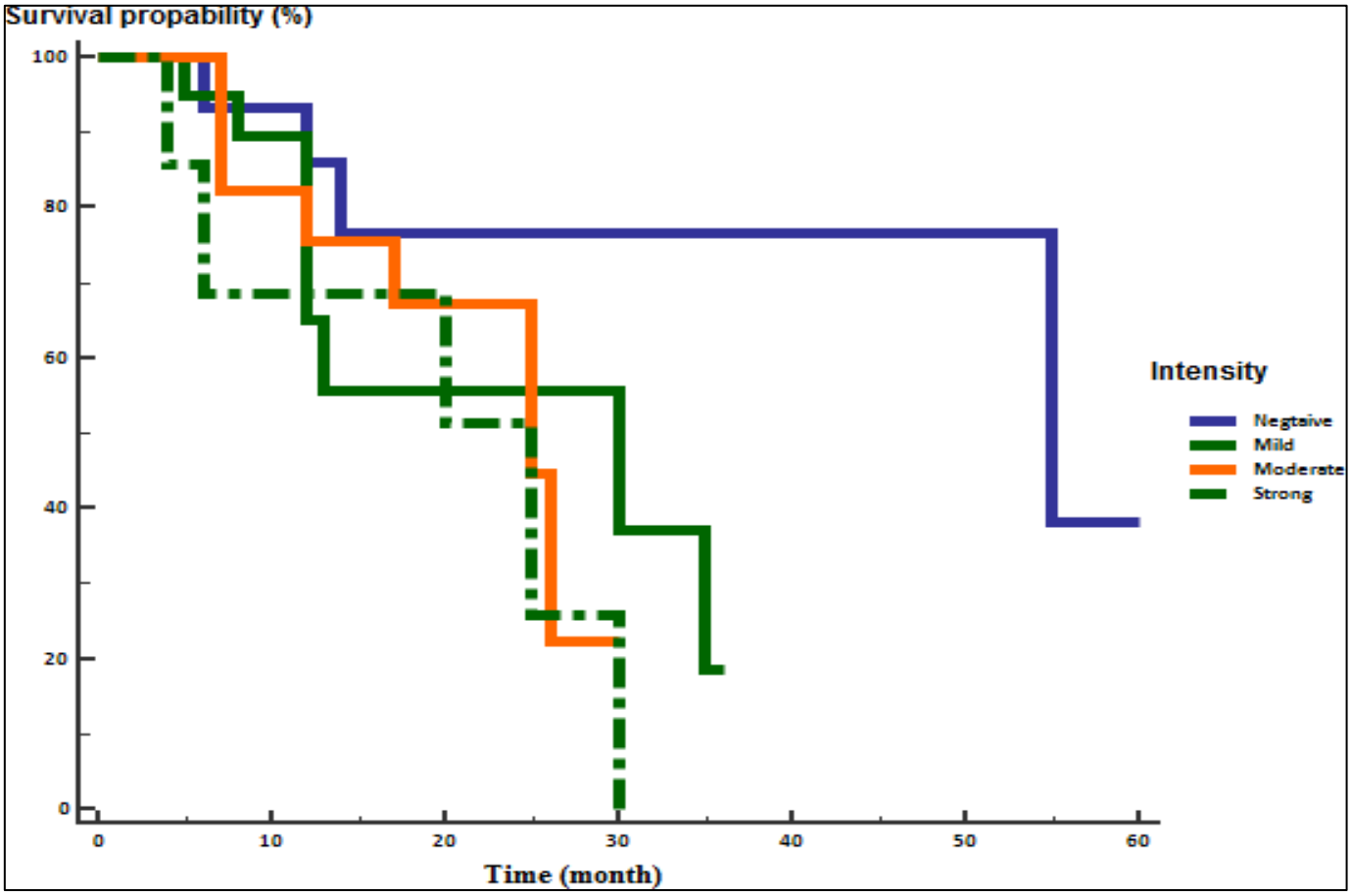

Figure (2): Overall survival based on the intensity of FGL2.

Accuracy of FGL2 in the prediction of progression and mortality among the studied patients with high-grade glioma (Table 6):

For prediction of progression, FGL2 at a cut-off point > 60% has 92.3% sensitivity and 28% specificity with overall accuracy was 42.1%. For prediction of mortality, FGL2 at a cut-off point > 70% has 67% sensitivity and 61% specificity with overall accuracy being 63.4%.

Table (6): Accuracy of FGL2 in the prediction of progression and survival

Indices	Progression	Mortality
Sensitivity	92.3%	67%
Specificity	28%	61%
Cutoff point	> 60%	> 70%
Negative predictive value	96%	73%
Accuracy	42.1%	63.4%
Area under curve	0.78	0.65
P value	**0.04**	**0.03**

Data expressed as mean (95% confidence interval). P-value was significant if < 0.05. FGL2: fibrinogen-like protein-2

DISCUSSION

Immunosuppressive effectors, such as FoxP3+ regulatory T cells, M2 macrophages, immunosuppressive cytokines, immunological checkpoints, myeloid-derived suppressor cells (MDSCs), and tumor-associated microglia/ macrophages (TAMs), interfere with the response to immunotherapy in glioblastoma. FoxP3 is required for FGL2 to function as a Treg effector [3, 6], which controls immunity [7]. In numerous models, FGL2 has been linked to the inhibition of Th1-polarized immune responses [8, 9, 10].The mean age of enrolled patients was 48.88 ± 12.45 years in our study but GBM is primarily diagnosed at an older age; the median diagnosis at 64 years old as reported by **Young *et al.*** [11]. Majority (75%) of patients were males and (25%) patients were females in our study which agreed with Cantrell et al where GBM 1.6 higher in males compared by females[12].

Contrary to what we discovered in our study, **Chakrabarti *et al.*** [13] reported that the supratentorial region (frontal, temporal, parietal, and occipital lobes) is where GBM is most frequently discovered, with the frontal lobe having the highest incidence and multiple lobes (overlapping tumors) having the second-and third-highest incidences, respectively. The most frequently injured areas were the parietal and temporal lobes (28.3% and 21.7%, respectively).

Latha *et al.* [14] examined the role of FGL2 overexpression using an animal model to ascertain its impact on tumor development [9]. The study demonstrates elevated FGL2 expression in surgically removed tumors that developed from low to high

grade. GBM cases with overexpression of FGL2 (n = 195) had statistically significantly shorter survival (median = 62.9 months) compared to cases with low expression (n = 325, median = 94.4 months), P=001. GBM cases with overexpression of FGL2 (n = 195) had statistically significantly shorter survival (median = 62.9 months) compared to cases with low expression (n = 325, median = 94.4 months).

CONCLUSION

FGL2 expression in high-grade glioma patients can be utilized as a prognostic indicator, though further research is needed to fully understand the predictive usefulness.

Financial support and sponsorship: This work supported by Faculty of Medicine Assiut University.
Conflict of interest: Nil.

REFERENCES

1. **Hanif F, Muzaffar K, Perveen K *et al.* (2017):** Glioblastoma Multiforme: A Review of its Epidemiology and Pathogenesis through Clinical Presentation and Treatment. Asian Pacific Journal of Cancer Prevention, 18(1):3-9.
2. **Zhu P, Du X, Lu G *et al.* (2017):** Survival benefit of glioblastoma patients after FDA approval of temozolomide concomitant with radiation and bevacizumab: a population-based study. Oncotarget., 8(27):44015-18.
3. **Zheng Y, Josefowicz S, Kas A *et al.* (2007):** Genome-wide analysis of Foxp3 target genes in developing and mature regulatory T cells. Nature, 445(7130):936-40.
4. **Hu J, Yan J, Rao G *et al.* (2016):** The duality of Fgl2-secreted immune checkpoint regulator versus membrane-associated procoagulant: therapeutic potential and implications. International Reviews of Immunology, 35(4):325- 39.
5. **Yan J, Kong L, Hu J *et al.* (2015):** FGL2 as a multimodality regulator of tumor-mediated immune suppression and therapeutic target in gliomas. Journal of the National Cancer Institute, 107(8):65-8.
6. **Williams L, Rudensky A (2007):** Maintenance of the Foxp3-dependent developmental program in mature regulatory T cells requires continued expression of Foxp3. Nat Immunol., 8(3):277–284.
7. **Chan C, Kay L, Khadaroo R *et al.* (2003):** Soluble fibrinogen-like protein 2/fibroleukin exhibits immunosuppressive properties: Suppressing T cell proliferation and inhibiting maturation of bone marrow-derived dendritic cells. J Immunol., 170(8):4036–4044.
8. **Shalev I, Wong K, Foerster K *et al. et al.* (2009):** The novel CD4+CD25+ regulatory T cell effector molecule fibrinogen-like protein 2 contributes to the outcome of murine fulminant viral hepatitis. Hepatology, 49(2):387–397.
9. **Khattar R, Luft O, Yavorska N *et al.* (2013):** Targeted deletion of FGL2 leads to increased early viral replication and enhanced adaptive immunity in a murine model of acute viral hepatitis caused by LCMV WE. PLoS One, 8(10):e72309.
10. **Joller N, Lozano E, Burkett P *et al.* (2014):** Treg cells expressing the coinhibitory molecule TIGIT selectively inhibit proinflammatory Th1 and Th17 cell responses. Immunity, 40(4):569–581.
11. **Young J, Chmura S, Wainwright D *et al.* (2017):** Management of glioblastoma in elderly patients. Journal of the Neurological Sciences, 380(7):250-5.
12. **Cantrell J, Waddle M, Rotman M *et al.* (2019):** Progress toward long-term survivors of glioblastoma. Mayo Clinic Proceedings, 94(7):1278-86.
13. **Chakrabarti I, Cockburn M, Cozen W *et al.* (2005):** A population- based description of glioblastoma multiforme in Los Angeles County, 1974–1999. Cancer: Interdisciplinary International Journal of the American Cancer Society, 104(12):2798-806.
14. **Latha K, Yan J, Yang Y *et al.* (2019):** The role of fibrinogen-like protein 2 on immunosuppression and malignant progression in glioma. Journal of the National Cancer Institute, 111(3):292-300.